# IN VIVO SCREENING OF CANDIDATE PRETREATMENT COMPOUNDS AGAINST CYANIDE USING MICE



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## ABSTRACT

An in vivo screening procedure was established at Battelle's Medical Research and Evaluation Facility (MREF) to evaluate the efficacy of candidate pretreatment compounds in mice challenged with the blood agent, sodium cyanide (NaCN). Male albino mice of ICR outbred stock weighing between 22.5 and 27.5 g are challenged by intramuscular (i.m.) injection, at a volume of 0.5 mL/kg, of a dose of NaCN twice the $LD_{50}$ of untreated mice as determined on that day of testing. Candidate drugs are tested at fractions of their $LD_{50}$ or their limit of solubility in the most optimum vehicle and given intraperitoneally (i.p.) to separate groups of mice at either 60 or 15 min prior to NaCN challenge. Sodium thiosulfate (1000 mg/kg)/sodium nitrite (100 mg/kg) controls are injected i.p. only at 60 min prior to challenge. A test compound is deemed effective if, at any of three concentrations tested, or at either pretreatment time, it is statistically more efficacious in preventing lethality than is a negative control substance (candidate compound vehicle). Since 1988, approximately 250 candidate compounds have been evaluated with 22 of these passing the efficacy screen. Motor incapacitation is tested in survivors of efficacy experiments 24 hr following NaCN injection using a horizontal screen test.

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## INTRODUCTION

The Drug Assessment Division of the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) is responsible for evaluating candidate pretreatment compounds and identifying those which are safe and effective in preventing or reversing the effects of cyanide intoxication. Battelle's MREF, as part of its tasking to evaluate efficacy of candidate pretreatment and therapeutic compounds against a Chemical Surety Materiel (CSM) challenge for the Drug Assessment Division, has established a large-scale screen for evaluating the ability of compounds to counter cyanide intoxication.

## MATERIALS

Four-week-old, male albino mice of the ICR outbred strain (Charles River Laboratories, Portage, Michigan) are group-caged and quarantined (with pelleted Purina Rodent Chow and water available ad libitum) for a minimum of three days before being placed on study. Following injections, animals are housed individually in polycarbonate cages on stainless steel racks with automatic watering devices for the 24-hr duration of the study. Only mice weighing between 22.5 and 27.5 g are used, and all mice are fasted three hr prior to the i.p. injection of pretreatment compounds. Survivors of studies are euthanatized using a carbon dioxide chamber.

All test compounds provided to Battelle by the Basic Assessment Branch, Drug Assessment Division, USAMRICD are submitted to the MREF through the Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR). Compound shipments are followed by Test Assignment Sheets, assigning the tests to be performed with each compound using specific protocols. Compounds are stored in amber dasiccator cabinets, or in clear desiccator cabinets if refrigeration or freezing temperatures are required.

Sodium cyanide (Aldrich Chemical Company) solutions are prepared on each day of study, using 0.9 percent saline to make a 30 mg/mL stock solution. Further dilutions are prepared, using the USAMRICD Real Time Animal Room Data Collection (ARDC) system to calculate dilution factors, to provide dosing solutions for determining the median lethal dose of i.m. administered NaCN. The ARDC computer system is also used to randomize animal treatments, and analyze, document, and archive the acute toxicity and efficacy results of candidate compounds in this screen.

## METHODS

Screening procedures include the performance of candidate compound solubility testing, range-finding acute toxicity and more definitive $LD_{50}$ determinations for the test compounds, and evaluations for efficacy in countering the effects of exposure to NaCN. Solubility determinations for the test compounds are accomplished using a series

of preference-ordered solvents until suitably concentrated solutions are obtained. Any compound found to have a solubility <10 mg/mL in all designated vehicles is suspended in 0.5% methylcellulose at a 100 mg/mL concentration for testing.

A range-finding acute candidate compound toxicity determination in mice, based on the limit of solubility of the compound, is conducted to establish the approximate $LD_{50}$ of each compound. To conserve the limited quantities of compound available, dose selection procedures and the number of animals dosed are conservative. If lethality is not observed within 24 hr of injection of the test compound at its limit of solubility or at a specified maximum dose of 1000 mg/kg, a more definitive $LD_{50}$ study is not performed. If all treated animals are killed by injection of the test compound, further range-finding studies are required. If some, but not all, of the animals are killed during these studies, a more definitive $LD_{50}$ experiment is performed to estimate more accurately the dose that will kill, within 24 hr, 50 percent of the animals injected. Survivors of definitive $LD_{50}$ testing are evaluated for motor incoordination using a horizontal screen test.

For daily agent potency evaluations, NaCN solutions with concentrations of 8.0, 6.2, 5.0, 4.0, and 3.2 mg/mL are made from a freshly prepared 30 mg/mL stock solution using the ARDC computer system to calculate dilutions. Six mice are dosed (using ARDC randomization) i.m. in the right hind limb with each NaCN concentration at 0.5 mL/kg. Based on lethality 30 min after $LD_{50}$ mice are dosed, the ARDC system is used to compute the NaCN $LD_{50}$, and efficacy study mice are then dosed at 2 x $LD_{50}$. If the calculated 30-min $LD_{50}$ in a study falls outside the 95 percent confidence limits of the historic mean $LD_{50}$ (2.7 ± 1.1 mg/kg), that solution of NaCN is not used for efficacy testing. A fresh stock solution is made and an agent $LD_{50}$ test performed again. At the end of efficacy testing, an additional 15 mice (5 doses with 3 mice per dose) are injected with the various concentration solutions prepared for agent potency testing to assure stability of the NaCN solutions.

Three candidate compound doses are tested for efficacy in counteracting NaCN intoxication, using fractions of the test compound $LD_{50}$ or fractions of its limit of solubility in the most optimum vehicle. All candidate pretreatment compounds are given i.p., using a dosing volume of 5 mL/kg, to groups of ten mice for each candidate compound dose injected 60 min or at 15 min prior to NaCN injection. Two groups of ten mice each serve as negative controls and are treated at either 60 or 15 min prior to challenge with 5 mL/kg i.p. of the vehicle used in test compound solutions. Ten positive control mice are given 5 mL/kg of a sodium thiosulfate (200 mg/mL)/sodium nitrite (20 mg/mL) solution i.p. at 60 min prior to NaCN challenge.

All efficacy test animals are evaluated at 24 hr following NaCN dosing. The number of living animals in each group is recorded and survivors are tested for motor incoordination or incapacitation using a

horizontal screen test. Mice which climbed to the top of a screen grid within 1 min of inversion pass the test; mice which fall or cling to the bottom of the grid for the 1-min test period fail.

## RESULTS

Results of candidate efficacy studies are archived using the ARDC system and electronically sent to USAMRICD for weekly review. A test compound is considered to be effective and passes the initial screen if, at any of the three concentrations tested, or at either pretreatment time, it is statistically more efficacious in preventing lethality than is the negative control substance (candidate compound vehicle). Initial anti-cyanide compound testing at the MREF, through September 1992, identified 22 compounds out of the 250 evaluated that were efficacious, and these are listed in Table 1.

TABLE 1. EFFICACIOUS ANTI-CYANIDE COMPOUNDS AS DETERMINED FROM INITIAL IN VIVO SCREENING RESULTS AT MREF

| ICD Compound | Solvent | Date of Study | NaCN $LD_{50}$ mg/kg | Pretreatment Doses mg/kg | # Survivors/# Dosed | |
|---|---|---|---|---|---|---|
| | | | | | 60 min | 15 min |
| 1021 | Multisol | 8/31/89 | 2.5 | negative control | 1/10 | 2/10 |
| BL52061 | | | | 6.7 | 1/10 | 1/10 |
| | | | | 26.9 | 1/10 | 4/10 |
| | | | | 107.5 | 7/10 | 8/10 |
| | | | | positive control | 10/10 | - |
| 1022 | Multisol | 8/31/89 | 2.5 | negative control | 1/10 | 2/10 |
| BL52098 | | | | 5.16 | 1/10 | 0/10 |
| | | | | 20.6 | 1/10 | 3/10 |
| | | | | 82.5 | 0/10 | 8/10 |
| | | | | positive control | 10/10 | - |
| 1104 | Water | 9/29/88 | 3.6 | negative control | 0/10 | 0/10 |
| BL40230 | | | | 3.0 | 0/10 | 0/10 |
| | | | | 12.2 | 2/10 | 0/10 |
| | | | | 48.9 | 9/10 | 9/10 |
| | | | | positive control | 9/10 | - |
| 1115 | EtOH/PG | 11/8/88 | 2.7 | negative control | 0/10 | 0/10 |
| BL40123 | | | | 12.0 | 1/10 | 2/10 |
| | | | | 48.5 | 2/10 | 4/10 |
| | | | | 194.0 | 1/10 | 3/10 |
| | | | | positive control | 10/10 | - |
| 1171 | Water | 10/19/88 | 2.8 | negative control | 1/10 | 0/10 |
| BL40249 | | | | 4.7 | 0/10 | 3/10 |
| | | | | 18.7 | 6/10 | 9/10 |
| | | | | 75.0 | 10/10 | 10/10 |
| | | | | positive control | 10/10 | - |
| 1297 | PEG | 9/12/89 | 2.6 | negative control | 0/10 | 0/10 |
| BL53228 | | | | 7.5 | 0/10 | 0/10 |
| | | | | 30.0 | 10/10 | 10/10 |
| | | | | 120.0 | 7/10 | 10/10 |
| | | | | positive control | 10/10 | - |

TABLE 1.
(Continued)

| ICD Compound | Solvent | Date of Study | NaCN $LD_{50}$ mg/kg | Pretreatment Doses mg/kg | # Survivors/# Dosed 60 min | 15 min |
|---|---|---|---|---|---|---|
| 1297* | PEG | 2/13/92 | 2.9 | negative control | 1/10 | 0/10 |
| BM08666 | | | | 13.0 | 0/10 | 3/10 |
| | | | | 52.5 | 10/10 | 10/10 |
| | | | | 210.0 | 5/10 | 5/10 |
| | | | | positive control | 10/10 | - |
| 1350 | Water | 9/11/89 | 2.5 | negative control | 0/10 | 1/10 |
| BJ08241 | | | | 2.1 | 0/10 | 0/10 |
| | | | | 8.5 | 0/10 | 0/10 |
| | | | | 34.0 | 1/10 | 5/10 |
| | | | | positive control | 10/10 | - |
| 1584 | EtOH/PEG | 6/19/90 | 3.0 | negative control | 0/10 | 0/10 |
| BM01836 | | | | 4.1 | 0/10 | 0/10 |
| | | | | 16.5 | 0/10 | 0/10 |
| | | | | 66.0 | 0/10 | 8/10 |
| | | | | positive control | 10/10 | - |
| 1585 | PEG | 6/20/90 | 3.1 | negative control | 0/10 | 0/10 |
| BM01845 | | | | 15.0 | 0/10 | 0/10 |
| | | | | 60.0 | 2/10 | 2/10 |
| | | | | 240.0 | 2/10 | 6/10 |
| | | | | positive control | 10/10 | - |
| 1587 | EtOH/PEG | 6/21/90 | 3.7 | negative control | 0/10 | 2/10 |
| BM01863 | | | | 1.15 | 0/10 | 2/10 |
| | | | | 4.6 | 1/10 | 1/10 |
| | | | | 18.5 | 3/10 | 6/10 |
| | | | | positive control | 10/10 | - |
| 1738 | EtOH/$H_2O$ | 11/28/90 | 2.9 | negative control | 6/10 | 4/10 |
| AW02325 | | | | 4.0 | 0/10 | 5/10 |
| | | | | 16.0 | 2/10 | 7/10 |
| | | | | 64.2 | 6/10 | 10/10 |
| | | | | positive control | 10/10 | - |

TABLE 1.
(Continued)

| ICD Compound | Solvent | Date of Study | NaCN $LD_{50}$ mg/kg | Pretreatment Doses mg/kg | # Survivors/# Dosed 60 min | 15 min |
|---|---|---|---|---|---|---|
| 1738* | EtOH/$H_2O$ | 10/21/92 | 2.7 | negative control | 0/10 | 1/10 |
| AW02325 | | | | 4.0 | 0/10 | 0/10 |
| | | | | 16.0 | 0/10 | 0/10 |
| | | | | 64.2 | 0/10 | 5/10 |
| | | | | positive control | 10/10 | - |
| 1791 | EtOH/PEG | 10/25/90 | 2.6 | negative control | 0/10 | 1/10 |
| BM05638 | | | | 9.38 | 0/10 | 9/10 |
| | | | | 37.5 | 9/10 | 10/10 |
| | | | | 150.0 | 10/10 | 9/10 |
| | | | | positive control | 10/10 | - |
| 1791* | EtOH/PEG | 2/12/92 | 2.7 | negative control | 0/10 | 1/10 |
| BM08906 | | | | 11.5 | 1/10 | 7/10 |
| | | | | 46.25 | 10/10 | 10/10 |
| | | | | 185.0 | 9/10 | 10/10 |
| | | | | positive control | 10/10 | - |
| 1816 | Multisol | 11/29/90 | 2.6 | negative control | 0/10 | 0/10 |
| BM06046 | | | | 12.5 | 0/10 | 0/10 |
| | | | | 50.0 | 1/10 | 0/10 |
| | | | | 200.0 | 5/10 | 4/10 |
| | | | | positive control | 10/10 | - |
| 1824 | Multisol | 12/3/90 | 2.6 | negative control | 0/10 | 0/10 |
| BM06126 | | | | 4.4 | 0/10 | 1/10 |
| | | | | 17.75 | 0/10 | 4/10 |
| | | | | 71.0 | 0/10 | 2/10 |
| | | | | positive control | 10/10 | - |
| 1907 | Water | 4/2/91 | 2.7 | negative control | 1/10 | 0/10 |
| BM07230 | | | | 3.8 | 0/10 | 1/10 |
| | | | | 15.3 | 1/10 | 1/10 |
| | | | | 61.0 | 0/10 | 4/10 |
| | | | | positive control | 10/10 | - |

TABLE 1.
(Continued)

| ICD Compound | Solvent | Date of Study | NaCN $LD_{50}$ mg/kg | Pretreatment Doses mg/kg | # Survivors/# Dosed | |
|---|---|---|---|---|---|---|
| | | | | | 60 min | 15 min |
| 2109 | HCl | 1/30/92 | 2.6 | negative control | 0/10 | 0/10 |
| BM09501 | | | | 14.8 | 1/10 | 1/10 |
| | | | | 59.4 | 1/10 | 1/10 |
| | | | | 237.5 | 2/10 | 4/10 |
| | | | | positive control | 9/10 | - |
| 2193 | Methylcellulose | 2/11/92 | 2.4 | negative control | 2/10 | 1/10 |
| BM10273 | | | | 15.6 | 6/10 | 9/10 |
| | | | | 62.5 | 10/10 | 9/10 |
| | | | | 250.0 | 10/10 | 10/10 |
| | | | | positive control | 10/10 | - |
| 2194 | Methylcellulose | 2/11/92 | 2.4 | negative control | 2/10 | 1/10 |
| BM09636 | | | | 15.6 | 5/10 | 9/10 |
| | | | | 62.5 | 8/10 | 9/10 |
| | | | | 250.0 | 9/10 | 9/10 |
| | | | | positive control | 10/10 | - |
| 2195 | PEG | 2/13/92 | 2.9 | negative control | 1/10 | 0/10 |
| BM08817 | | | | 1.2 | 0/10 | 0/10 |
| | | | | 4.9 | 0/10 | 10/10 |
| | | | | 19.75 | 10/10 | 10/10 |
| | | | | positive control | 10/10 | - |
| 2196 | EtOH/PEG | 2/12/92 | 2.7 | negative control | 0/10 | 1/10 |
| BM08586 | | | | 15.6 | 4/10 | 10/10 |
| | | | | 62.5 | 10/10 | 9/10 |
| | | | | 250.0 | 2/8 | 2/8 |
| | | | | positive control | 10/10 | - |
| 2214 | EtOH/$H_2O$ | 6/2/92 | 2.5 | negative control | 0/10 | 0/10 |
| BM10755 | | | | 4.5 | 0/10 | 0/10 |
| | | | | 17.8 | 0/10 | 0/10 |
| | | | | 71.3 | 1/10 | 10/10 |
| | | | | positive control | 9/10 | - |

TABLE 1.
(Continued)

| ICD Compound | Solvent | Date of Study | NaCN $LD_{50}$ mg/kg | Pretreatment Dose mg/kg | # Survivors/# Dosed | |
|---|---|---|---|---|---|---|
| | | | | | 60 min | 15 min |
| 2240 | EtOH/$H_2O$ | 6/2/92 | 2.5 | negative control | 0/10 | 0/10 |
| BM11109 | | | | 2.3 | 0/10 | 0/10 |
| | | | | 9.3 | 1/10 | 0/10 |
| | | | | 37.0 | 1/10 | 5/10 |
| | | | | positive control | 9/10 | - |

*Some candidate compounds were repeated in the complete screening process.

## CONCLUSION

This in vivo screening procedure has identified a number of compounds which counter the effects of cyanide intoxication in mice. This information is of great value in determining which compounds warrant further evaluation.

## REFERENCES

1. Olson, C.T., Kiser, R.C., and Dill, G.S. 1992. Task 89-01: Screening of Candidate Pretreatment and Therapeutic Compounds in In Vivo Models. Report for USAMRICD under Contract DAMD17-89-C-9050.